Siwu decoction attenuates oxonate-induced hyperuricemia and kidney inflammation in mice

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[ABSTRACT] The aim of the study was to investigate the effects of Siwu decoction on hyperuricemia, kidney inflammation, and dysfunction in hyperuricemic mice. Siwu decoction at 363.8, 727.5, and 1455 mg·kg$^{-1}$ was orally administered to potassium oxonate-induced hyperuricemic mice for 7 days. Serum urate, creatinine, and blood urea nitrogen levels and hepatic xanthine oxidase (XOD) activity were measured. The protein levels of hepatic XOD and renal urate transporter 1 (URAT1), glucose transporter 9 (GLUT9), organic anion transporters 1 (OAT1), ATP-binding cassette subfamily G member 2 (ABCG2), organic cation transporter 1 (OCT1), OCT2, organic cation/carnitine transporter 1 (OCTN1), OCNT2, Nod-like receptor family, pyrin domain containing 3 (NLRP3), apoptosis-associated speck-like protein (ASC), Caspase-1, and interleukin-1$\beta$ (IL-1$\beta$) were determined by Western blotting. Renal histopathology change was obtained following hematoxylin-eosin staining. Our results indicated that Siwu decoction significantly reduced serum urate, creatinine and blood urea nitrogen levels and increased fractional excretion of uric acid in hyperuricemic mice. It effectively reduced hepatic XOD activity and protein levels in this animal model. Furthermore, Siwu decoction down-regulated URAT1 and GLUT9 protein levels, and up-regulated the protein levels of OAT1, ABCG2, OCT1, OCT2, OCTN1, and OCTN2 in the kidney of the hyperuricemic mice. Additionally, Siwu decoction remarkably reduced renal protein levels of NLRP3, ASC, Caspase-1, and IL-1$\beta$ in the hyperuricemic mice. These results suggested that Siwu decoction exhibited anti-hyperuricemic and anti-inflammatory effects by inhibiting hepatic XOD activity, regulating renal organic ion transporter expression, and suppressing renal NLRP3 inflammasome activation, providing the evidence for its use in the treatment of hyperuricemia and associated kidney inflammation.

[KEY WORDS] Siwu decoction; Hyperuricemia; Renal organic ion transporter; NLRP3 inflammasome; Kidney inflammation

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Introduction

Hyperuricemia is an important risk factor for the development of gout, insulin resistance, coronary heart disease, diabetes, and metabolic syndrome$^{[1-3]}$. Generally, xanthine oxidase (XOD) oxidizes xanthine to uric acid mainly in liver, through the purine metabolic pathway; its hyperactivity causes high levels of serum uric acid$^{[4]}$. Increasing evidence supports the notion that kidney urate transport-related proteins mediate kidney urate excretion to maintain blood urate balance$^{[5-9]}$. Among them, urate transporter 1 (URAT1) and glucose transporter 9 (GLUT9) regulate kidney uric acid reabsorption$^{[5-7]}$, while organic anion transporter 1 (OAT1) and native ATP-binding cassette subfamily G member 2 (ABCG2) regulate renal urate secretion$^{[8-9]}$. Of note, dysregulation of these urate transporters alters urate handling in humans$^{[10-11]}$.

Hyperuricemia aggravates kidney dysfunction. Organic cation transporter 1 (OCT1), OCT2, organic cation and carnitine transporter 1 (OCTN1), and OCNT2 mediate the excretion of organic cations and carnitine in renal proximal tubules$^{[7]}$. Down-regulation of these kidney organic ion transporters may increase the risk for kidney dysfunction in hyperuricemia$^{[12-14]}$. On the other hand, the elevated serum urate levels cause kidney inflammation$^{[15]}$. Nod-like receptor family, pyrin domain containing 3 (NLRP3), interacts with the bridging molecule apoptosis-associated speck-like protein (ASC) to activate caspase-1, leading to mature interleukin-1$\beta$ (IL-1$\beta$) production$^{[16-17]}$. Uric acid as a proinflammatory molecule activates the NLRP3 inflammasome, causing...
inflammatory response \cite{16}. Therefore, regulation of renal organic ion transporter system and suppression of the NLRP3 inflammasome activation may reduce serum urate levels and alleviate kidney inflammation and dysfunction in hyperuricemia.

Siwu decoction, composed of Angelica sinensis radix (Angelica sinensis (Oliv. Diels), Chuanxiong rhizome (Ligusticum chuanxiong Hort.), Paeonia radix alba (Paeonia lactiflora Pall) and Rehmanniae radix praeparata (Rehmannia glutinosa Libosch.), has long been used in traditional Chinese medicine. According to Xian-shou-li-shang-xu-duan-mi-fang in Tang dynasty and Tai-ping-hui-min-he-ji-ju-fang in Song dynasty, Siwu decoction is prescribed to alleviate trauma and ecchymosis, promote blood circulation, and treat gynecological and obstetrical diseases \cite{18-19}. The combination of Siwu and Ermiao decoction is reported to markedly decrease serum uric acid levels and inhibit hepatic XOD activity in hyperuricemic rats \cite{20}. A recent study shows that Siwu decoction inhibits triphasic skin reaction in passively sensitized mice with inflammation \cite{21}. In the present study, we investigated the effects and possible mechanisms of Siwu decoction on hyperuricemia, renal inflammation, and dysfunction in the potassium oxonate-induced hyperuricemic mice.

Materials and Methods

Chemicals and reagents

Potassium oxonate, allopurinol, hematoxylin & eosin reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). The ingredients of Siwu decoction, including Angelica sinensis granule (batch number 1303102, 8 g), ligusticum chuanxiong granule (batch number 1304033, 8 g), paeonia lactiflora granule (batch number 1302031, 4 g) and, rehmannia glutinosa granule (batch number 1309107, 8 g), were purchased from Jiangyin Tianjiang Pharmaceutical Co., Ltd. (Jiangyin, China).

Standards of ferulic acid, paeoniflorin, and ligustrazine hydrochloride were obtained from National Institutes for Food and Drug Control (Beijing, China). Assay kits for uric acid, creatinine, blood urea nitrogen (BUN), and XOD activity were purchased from Jiancheng Biotech (Nanjing, China). Rabbit anti-mouse XOD and Caspase-1 antibodies and mouse anti-β-actin monoclonal antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Rabbit anti-mouse URAT1, GLUT9, OCT1, and OCT2 antibodies were obtained from Cellchip Biotech (Beijing, China). Rabbit anti-mouse OCTN1 and OCTN2 antibodies were purchased from Alpha Diagnostic International, Inc. (San Antonio, TX, USA). Rabbit anti-mouse OAT1 antibody was also from Cellchip Biotech. Rabbit anti-mouse ABCG2 antibody was purchased from Cell Signaling Technology, Inc. (Boston, MA, USA). Rabbit anti-mouse NLRP3, ASC, and IL-1β antibodies were purchased from Abcam (Cambridge, MA, USA). Mouse GAPDH monoclonal antibody was from Kangcheng Biotech (Shanghai, China). All other chemicals used in the present study were commercial products of the highest purity available.

UPLC-MS analysis of Siwu decoction

Qualitative and quantitative analyses of ferulic acid, paeoniflorin, and ligustrazine in Siwu decoction were accomplished using ultra performance liquid chromatography (UPLC)-Mass spectrometric (MS) analysis. UPLC was performed on a Waters ACQUITY UPLC™ system (Waters Corporation, Milford, MA, USA) equipped with a binary solvent delivery system and autosampler. The chromatography was performed on a Waters ACQUITY UPLC BEH C18 (2.1 mm × 100 mm, 1.7 μm). The mobile phase consisted of the following 22 min sequence of linear gradients and an isoocratic flow of 0.1% formic acid-water (solvent A) balanced with solvent B (0.1% acetonitrile) at a flow rate of 0.4 mL·min⁻¹ under the condition of column temperature 30 °C: 0–2 min, 95% A, 5% B; 2–13 min, 95%–60% A, 5%–40% B; 13–17 min, 60%–20% A, 40%–80% B; 17–19 min, 20%–5% A, 80%–95% B; 19–20 min, 5% A, 95% B; 20–21 min, 5%–95% A, 95%–5% B; and 21–22 min, 95% A, 5% B. The injection volume for all analyses was 10 μL.

MS analysis was carried out with a Waters ACQUITY Synapt Q-TOF mass spectrometer (Waters MS Technologies, Manchester, UK) connected to the Waters ACQUITY UPLC system via electrospray ionization (ESI) interface. High purity nitrogen was used as the nebulizer and auxiliary gas, and argon was the collision gas. The Q-TOF MS was operated in negative ion mode with a capillary voltage of 3 kV, a sampling cone voltage of 45 V, a desolvation temperature of 500 °C, a source block temperature of 150 °C, a collision energy of 6–40 V, and a ion energy of 1 V.

Animals

Male Kun-Ming mice (weighing 20 ± 2 g) were purchased from the Laboratory Animal Center of Academy of Military Medical Sciences (Beijing, China, Certificate NO. SCXK- (Military) 2012-0004) and housed in a same room in which the temperature was 25 ± 1 °C with relative humidity (55 ± 5)% and 12 h light/12 h dark cycles were maintained with the lights on at 7 : 00 a.m. They are given a standard chow and water ad libitum for the duration of the study. The mice were allowed to adapt to the environment for a week before being used for the experiment. All studies were performed with the standards established by the Institutional Animal Care Committee at the Nanjing University and the China Council on Animal Care at the Nanjing University (The Ministry of Science and Technology of the People’s Republic of China, 2006), and experiment number approved by Institutional Animal Care Committee is 201305620.

Hyperuricemic mice and drug administration

Hyperuricemia was induced with uricase inhibitor potassium oxonate (250 mg·kg⁻¹) in mice as described in our previous report \cite{22}. According to the Tai-ping-hui-min-he-ji-ju-fang in Song dynasty and Traditional Chinese Medicine Lexicon Attachment, the dosage of Siwu decoction for adults is 11.19 g·d⁻¹ (the total raw materials), and the equivalent mouse dosage is 1 455 mg·kg⁻¹·d⁻¹ as calculated by the
conversion of human dosage into that of mouse equivalent dose according to the respective body surface areas. The hyperuricemic mice were randomly divided into 5 experimental groups (10/group) receiving water (vehicle), Siwu decoction (363.8, 727.5 and 1 455 mg·kg⁻¹), and allopurinol (5 mg·kg⁻¹) daily, respectively. Siwu decoction and allopurinol were orally given at 9 : 00 a.m. on the day when potassium oxonate was given at 8 : 00 a.m.. They were administrated for 7 consecutive days.

**Urine, blood, and tissue sample collection**

24-h urine sample from each mouse was gathered in a metabolic cage and urine volume was recorded on the 7th day. All the mice had free access to standard chow and tap water in this metabolic cage. Urine samples were centrifuged at 2 000 × g for 10 min at 4 °C to remove the particulate contaminants. The supernatants were stored at −20 °C until uric acid and creatinine assays. On the seventh day, whole blood samples were collected 1 h after last drug administration. The blood was allowed to clot for approximately 1 h at room temperature and then centrifuged at 10 000 × g for 5 min to obtain the serum. The serum samples were stored at −20 °C until biochemical assays. Liver and parts of kidney tissues were rapidly and carefully removed on ice plate after mice sacrificed and immediately frozen in liquid nitrogen. These tissue samples were stored at −80 °C until Western blot assay. The remaining parts of renal tissues were fixed in 4 % formalin buffer for pathological analysis.

**Determination of uric acid, creatinine, and blood urea nitrogen**

Serum and urine levels of uric acid were determined using assay kit. Serum and urine creatinine levels were measured using trinitrophenol colorimetry kit. Fractional excretion of uric acid (FEUA) was calculated using the formula: FEUA = (urine uric acid × serum creatinine)/(serum uric acid × urine creatinine) × 100, expressed as percentage [23]. Serum BUN levels were determined using urease ultraviolet kit.

**Determination of hepatic XOD activity**

Hepatic XOD activity was measured using XOD assay kit following the manufacturer’s instruction.

**Histological study of renal tissues**

Kidney tissues with less than 5-mm thickness were fixed in 4% formalin buffer for 24 h. Then kidney tissues were dehydrated in increasing concentrations of alcohol in water. They were soaked in xylene after dehydrated, then embedded in paraffin before being cut into 5–8 μm sections. The sections were stained with hematoxylin & eosin reagent after dehydrated in decreasing concentrations of alcohol in water, and then mounted with neutral resin.

**Western blot analysis**

Protein extraction of kidney and liver tissues and Western blot analysis were performed using routine procedures described in our previous report [24]. The primary antibodies used were as follows: anti-XOD (1 : 500), or anti-URAT1 (1 : 1 000), anti-GLUT9 (1 : 1 000), anti-OAT1 (1 : 1 000), anti-ABCG2 (1 : 1 000), anti-OCT1 (1 : 1 000), anti-OCT2 (1 : 1 000), anti-OCTN1 (1 : 1 000), anti-OCTN2 (1 : 1 000), anti-NLRP3 (1 : 1 000), anti-ASC (1 : 1 000), anti-Caspase-1 (1 : 1 000), anti-IL-1β (1 : 1 000), anti-GAPDH (1 : 1 000) or anti-β-actin (1 : 5 000), respectively. The secondary antibody was mouse-anti-rabbit secondary antibody. Immunoreactive bands were quantified via measurement of the intensity of the signals with aid of ImageJ (National Institutes of Health). Relative quantitation for Western blot analysis was calculated after the normalization to the amount of mouse GAPDH or β-actin, respectively.

**Statistical analysis**

The data were expressed as the mean ± standard deviation (SD). Comparisons between groups were made using a one-way analysis of variance (ANOVA). *P < 0.05* was considered statistically significant. The figures were generated using the Statistical Analysis System (GraphPad Prism 5, GraphPad Software, Inc., San Diego, CA, USA).

**Results**

**Contents of ferulic acid, paeoniflorin and ligustrazine in Siwu decoction**

Ferulic acid, paeoniflorin and ligustrazine contents in Siwu decoction were analyzed by UPLC-MS method and quantified using each respective linear-regression line of standards. Accordingly, the contents of ferulic acid, paeoniflorin and ligustrazine in Siwu decoction were 0.323 ± 0.034 8, and 0.154 ± 3 μg·mg⁻¹, respectively.

**Effects of Siwu decoction on serum uric acid levels and kidney function in the hyperuricemic mice**

As expected, 250 mg·kg⁻¹ of potassium oxonate induced a significant increase in serum uric acid and creatinine levels in mice, compared with the normal vehicle control group. Siwu decoction at 727.5 and 1 455 mg·kg⁻¹ markedly reduced serum levels of uric acid and creatinine; the latter also restored alteration of urine uric acid and creatinine levels in hyperuricemic mice (Table 1). Siwu decoction at 363.8, 727.5 and 1 455 mg·kg⁻¹ significantly increased FEUA (Table 1) in hyperuricemic mice. Moreover, Siwu decoction at 1 455 mg·kg⁻¹ significantly reduced the serum BUN levels (*P < 0.001*) in the animals (Table 1). Allopurinol at 5 mg·kg⁻¹, as a positive control, showed similar effects in hyperuricemic mice (Table 1). These data indicated that Siwu decoction reduced hyperuricemia and improved kidney function in this animal model.

**Effects of Siwu decoction on liver XOD activity and protein levels in the hyperuricemic mice**

Hepatic XOD activity and protein levels were significantly increased in the hyperuricemic mice, compared with that in normal vehicle control group. Siwu decoction at 727.5 and 1 455 mg·kg⁻¹ effectively inhibited XOD activity and down-regulated hepatic XOD protein levels (Fig. 2) in the hyperuricemic mice. Allopurinol at 5 mg·kg⁻¹ also completely inhibited XOD activity and decreased XOD protein levels in this animal model. These data indicated that Siwu decoction inhibited hepatic XOD to reduce uric acid production in the hyperuricemic mice.
Table 1  Effects of Siwu decoction on parameters of serum uric acid levels and kidney function in the potassium oxonate-induced hyperuricemic mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg·kg⁻¹)</th>
<th>Urine in 24 h</th>
<th>Serum</th>
<th>FEUA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Uric acid (mg·dL⁻¹)</td>
<td>Creatinine (mg·dL⁻¹)</td>
<td>Uric acid (mg·dL⁻¹)</td>
</tr>
<tr>
<td>Normal</td>
<td>–</td>
<td>20.80 ± 1.53</td>
<td>26.07 ± 4.84</td>
<td>2.36 ± 0.51</td>
</tr>
<tr>
<td>Hyperuricemia</td>
<td>13.84 ± 2.44 **</td>
<td>18.43 ± 2.40 ***</td>
<td>3.76 ± 0.94 **</td>
<td>1.24 ± 0.13 ***</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>5</td>
<td>18.11 ± 2.00 **</td>
<td>24.64 ± 3.51</td>
<td>1.91 ± 1.07</td>
</tr>
<tr>
<td>Siwu decoction</td>
<td>363.8</td>
<td>15.25 ± 3.40</td>
<td>16.87 ± 1.15</td>
<td>3.17 ± 0.98</td>
</tr>
<tr>
<td></td>
<td>727.5</td>
<td>17.34 ± 2.18 **</td>
<td>21.19 ± 1.57</td>
<td>2.79 ± 0.63 **</td>
</tr>
<tr>
<td></td>
<td>1455</td>
<td>19.80 ± 1.85 ***</td>
<td>25.50 ± 1.24 **</td>
<td>2.15 ± 0.46 **</td>
</tr>
</tbody>
</table>

Each value represents mean ± SD (n = 8). Statistical analyses were performed by using a one-way ANOVA. FEUA: fraction excretion of uric acid. **P < 0.01, ***P < 0.001 vs normal vehicle group; *P < 0.05, **P < 0.01, ***P < 0.001 vs hyperuricemic vehicle group.

Fig. 1  Chromatographic profiles of Siwu decoction. Total ion chromatograms of Siwu decoction (A) and representative mass spectra of ferulic acid (B), paeoniflorin (C) and ligustazine (D), were analyzed by the UPLC-MS method.
Effects of Siwu decoction on hepatic XOD activity and protein levels in the hyperuricemic mice

Hepatic XOD activity (A) was measured using assay kit. Protein levels of hepatic XOD (B) were determined by Western blot analysis. Data represent mean ± SD from 6 mice/group. ##P < 0.01 vs normal vehicle group; *P < 0.05, ***P < 0.001 vs hyperuricemia vehicle group.

Fig. 2 Effects of Siwu decoction on hepatic XOD activity and protein levels in the hyperuricemic mice. Hepatic XOD activity (A) was measured using assay kit. Protein levels of hepatic XOD (B) were determined by Western blot analysis. Data represent mean ± SD from 6 mice/group. ##P < 0.01 vs normal vehicle group; *P < 0.05, ***P < 0.001 vs hyperuricemia vehicle group.

Effects of Siwu decoction on renal protein levels of URAT1, GLUT9, OAT1 and ABCG2 in the hyperuricemic mice

Renal URAT1 and GLUT9 protein levels were remarkably increased in hyperuricemic mice, compared with that in the normal group. Siwu decoction at 727.5 and 1455 mg kg⁻¹ effectively down-regulated renal protein levels of URAT1 and GLUT9 in this animal model (Figs. 3A-B). Compared to normal vehicle group, renal OAT1 and ABCG2

Fig. 3 Effects of Siwu decoction on renal URAT1, GLUT9, OAT1 and ABCG2 in the hyperuricemic mice. Renal protein levels of URAT1 (A), GLUT9 (B), OAT1 (C) and ABCG2 (D) were determined by Western blot analysis. Data represent mean ± SD from 6 mice/group. #P < 0.05, ##P < 0.01, ###P < 0.001 vs normal vehicle group; *P < 0.05, **P < 0.01, ***P < 0.001 vs hyperuricemia vehicle group.
protein levels were remarkably decreased in the hyperuricemic mice, which were restored effectively by Siwu decoction at 1,455 mg·kg⁻¹ (Figs. 3C-D). Allopurinol at 5 mg·kg⁻¹ down-regulated renal URAT1, OAT1 and ABCG2 protein levels, but failed to alter renal GLUT9 protein levels in this animal model (Fig. 3). These results indicated that Siwu decoction might have the potent uricosuric effects by regulating these renal urate transport-related proteins to promote kidney urate excretion in hyperuricemia.

**Effects of Siwu decoction on renal protein levels of OCT1, OCT2, OCTN1 and OCTN2 in the hyperuricemic mice**

Renal OCT1, OCT2, OCTN1 and OCTN2 protein levels were detected to investigate effects of Siwu decoction on kidney function in hyperuricemia. Compared with normal vehicle group, renal protein levels of OCT1, OCT2, OCTN1 and OCTN2 were significantly reduced in hyperuricemic mice (Fig. 4). Siwu decoction at 727.5 and 1,455 mg·kg⁻¹ remarkably up-regulated renal OCT1, OCT2 and OCTN2 protein levels (Figs. 4A-B, D), the latter significantly increased renal OCTN1 protein levels in hyperuricemic mice. It was also observed that renal protein levels of OCT1 and OCT2, as well as OCTN1 and OCTN2 were significantly increased in hyperuricemic mice treated with 5 mg·kg⁻¹ allopurinol (Fig. 4). These data indicated that Siwu decoction may regulate these renal organic cation transporters to improve kidney function in hyperuricemic mice.

![Fig. 4](image-url)  **Effects of Siwu decoction on renal OCT1, OCT2, OCTN1 and OCTN2 in the hyperuricemic mice.** Protein levels of renal OCT1 (A), OCT2 (B), OCTN1 (C) and OCTN2 (D) were determined by Western blot analysis. Data represent mean ± SD from 6 mice/group. ***P < 0.001 vs normal vehicle group; *P < 0.05, **P < 0.01 vs hyperuricemia vehicle group.

**Effects of Siwu decoction on renal inflammation in hyperuricemic mice**

Mild renal tubular dilatation and tubular epithelial cell vacuolar degeneration were observed in the hyperuricemic mice (Fig. 5B). Siwu decoction and allopurinol effectively improved these kidney histopathological changes in hyperuricemic mice (Figs. 5C, F). Furthermore, the hyperuricemic mice showed the increased renal protein levels of NLRP3, ASC, Caspase-1, and...
IL-1β (Fig. 6). Siwu decoction at 1,455 mg·kg⁻¹ and allopurinal at 5 mg·kg⁻¹ remarkably down-regulated renal NLRP3, ASC, Caspase-1, and IL-1β protein levels in the hyperuricemic mice (Fig. 6). These results suggested that Siwu decoction might suppress NLRP3 inflammasome to protect kidney function in the hyperuricemic mice.

Fig. 5  Siwu decoction improved kidney histopathological changes in the hyperuricemic mice. Original magnification × 200. Normal-vehicle mice (A); Hyperuricemia-vehicle mice (B); Hyperuricemia-vehicle mice treated with allopurinol at 5 mg·kg⁻¹ (C), Siwu decoction at 363.8 mg·kg⁻¹ (D), 727.5 mg·kg⁻¹ (E) and 1,455 mg·kg⁻¹ (F)

Fig. 6  Effects of Siwu decoction on renal NLRP3, ASC, Caspase-1 and IL-1β in the hyperuricemic mice. Renal protein levels of NLRP3 (A), ASC (B), Caspase-1 (C) and IL-1β (D) were determined by Western blot analysis. Data represent mean ± SD from 6 mice/group. *P < 0.05, **P < 0.01, ***P < 0.001 vs normal vehicle group; †P < 0.05, ‡P < 0.01, §§P < 0.001 vs hyperuricemia vehicle group
Discussion

Hyperuricemia is caused by over-generation and/or under-excretion of uric acid. Siwu decoction is a classical prescription of traditional Chinese medicine. Its combination with Ermiao decoction significantly reduces serum urate levels and inhibits hepatic XOD activity in hyperuricemic rats [20]. In the present study, Siwu decoction significantly decreased hepatic XOD activity and expression in the hyperuricemic mice, indicating that this decoction may block the synthesis of uric acid from purines. Of note, Siwu decoction obviously increased FEUA in hyperuricemic mice, indicating the enhancement of kidney urate excretion. Urate is reabsorbed via URAT1 on the tubular apical membrane. Absence of URAT1 gene mutations is detected in patients with primary gout [5, 25]. GLUT9 also affect renal uric acid reabsorption to influence blood uric acid levels over a wide range of values [26]. Renal OAT1 is functioned as secretion of urate on the basolateral membrane of renal proximal tubules [27]. ABCG2 located in the brush border membrane of kidney proximal tubule cells is a urate efflux transporter [9]. In the present study, Siwu decoction was found to down-regulate URAT1 and GLUT9 protein levels, and up-regulate OAT1 and ABCG2 protein levels in the kidney of the hyperuricemic mice. These results suggested that Siwu decoction had dual anti-hyperuricemic activity by inhibiting hepatic XOD to reduce uric acid production, and regulating renal urate transport-related proteins to promote renal urate excretion in hyperuricemia. To our best knowledge, this was the first report of Siwu decoction possibly targeting kidney URAT1, GLUT9, OAT1, and ABCG2.

Hyperuricemia is the key factor for the development of kidney inflammatory response and dysfunction [24-25]. Renal dysfunction is accompanied by an increase of serum creatinine and BUN. OCT1, OCT2, OCTN1, and OCTN2 are essential for kidney function of organic cations or/and carnitine transportation. In the present study, Siwu decoction reduced serum creatinine and BUN levels, and markedly up-regulated renal OCT1, OCT2, OCTN1 and OCTN2 protein levels in the hyperuricemic mice. These observations indicated that Siwu decoction might regulate these renal organic cation and carnitine transporters to remove the noxious substances accumulated in the kidney, showing its improvement of kidney function in hyperuricemia.

Hyperuricemia is associated with inflammation, which aggravates kidney dysfunction. High urate level is suggested to activate the NLRP3 inflammasome, resulting in the maturation of IL-1β in gout and pseudogout [28]. What’s important, the present study found that Siwu decoction markedly suppressed renal NLRP3 inflammasome activation to reduce IL-1β levels in hyperuricemic mice, being consistent with its alleviation of kidney histopathological change. These results suggested that Siwu decoction might inhibit inflammation to protect against kidney dysfunction in hyperuricemia.

Angelica sinensis polysaccharide possesses immunomodulatory action in mice [29]. Z-ligustilide and senkyunolide A from Ligusticum chuanxiong inhibit inflammatory cytokine production to reduce inflammation in rats [30]. Rehmannia glutinosa steamed root inhibits IL-1 secretion with anti-inflammatory activity in mouse astrocytes [31]. Total glucosides of paony lower blood lipids and suppress inflammatory cytokine expression in rats [32]. Paeoniflorin is also demonstrated to alleviate rat brain damage by suppressing neuroinflammatory reaction [32]. Ferulic acid is suggested to protect against various inflammatory diseases [33]. Ligustilide reduces ischemia-reperfusion-induced renal dysfunction in murine [34]. The underlying mechanisms by which ingredients of Siwu decoction possess anti-hyperuricemic and anti-inflammatory actions in hyperuricemia with kidney dysfunction need to be further explored.

In conclusion, Siwu decoction was demonstrated to have anti-hyperuricemic effects by inhibiting hepatic XOD to reduce uric acid production and mediating renal urate transport-related transporters to enhance kidney urate excretion in hyperuricemic mice. Furthermore, Siwu decoction was found to alleviate kidney inflammation and dysfunction by suppressing the NLRP3 inflammasome activation, and up-regulating organic cation and/carnitine transporters in hyperuricemic mice. The results from the present study may provide an evidence to support clinical therapeutic effects of Siwu decoction on hyperuricemia and kidney dysfunction.

References


